REMARKS

Claims 1-20 and 23-33 are pending in the application. Claims 21 and 22 have been cancelled herein without prejudice or disclaimer of the subject matter contained therein. Claims 1, 12-20, and 23 have been amended to correct typographical errors and issues relating to antecedent bases. No new matter has been introduced by these amendments, and entry of these amendments are respectfully requested.

The specification has been amended to correct various obvious typographical errors and to update the Cross-Reference to Related Applications to indicate that one parent application has issued as a patent. In particular, the insertion of the word "inverted" at page 11, line 26 and page 12, line 6 is obvious from the context of the paragraphs and is inserted for clarification purposes only. The change from "Table I" to "Figure 18" at pages 93, line 15 and page 94, line 26 is also obvious from the context of the paragraphs, the description of Figure 18 (herein amended) on page 20, and the data provided in Figure 18. Accordingly, no new matter has been introduced by way of these amendments, and entry of these amendments is respectfully requested.

The outstanding rejections are addressed individually below.

1. ***Corrected drawings have been provided.***

The drawings have been objected to for the reasons set forth on the form PTO-948. Replacement copies of the drawings containing corrections have been provided herewith. Accordingly, the Examiner is requested to reconsider and to withdraw this rejection.

2. ***Illegible text in the specification has been corrected.***

The specification has been amended to delete the illegible text at page 48, lines 27-28. Therefore, the Examiner is requested to reconsider and to withdraw this rejection.

3. Oath or declaration contains inventor's address.

The Examiner has objected to the oath or declaration because it does not identify the post office address of the inventor. A copy of the previously submitted declaration is herein provided (attached hereto as Exhibit A). As seen at the bottom of page 3 of the declaration, the inventor's post office address is included. Therefore, the Examiner is requested to reconsider and to withdraw this rejection.

4. The disclosure of parent application 08/532,979 provides sufficient support for the method claimed in the instant application.

Applicant has not been accorded the benefit of the earlier filing date of application 08/532,979 due to failure to provide sufficient support for the method claimed in the instant application. Applicant respectfully traverses this determination.

The Examiner opines that the parent application 08/532,979 does not provide specific guidance or support for practicing the claimed method comprising the use of an antibody that binds to EGFR or a cytotoxic agent selected from the group consisting of taxanes, platinum-derived agents, and topoisomerase II-selective drugs.

However, the parent application does provide support for the claimed method at page 27, line 28 to page 28, line 5. This paragraph, subsequently added to in the first continuation-in-part of the parent application and herein amended, also appears in the instant application at page 34, lines 20-33. The other therapies for cancer which were subsequently enumerated in the first continuation-in-part (cisplatin, carboplatin, paclitaxel, tamoxifen, taxol, interferon α and doxorubicin) as well as those specifically claimed in the instant application (the use of an antibody that binds to EGFR or a cytotoxic agent selected from the group consisting of taxanes, platinum-derived agents, and topoisomerase II-selective drugs) were well-known therapies as of the filing date of the parent application. These specifically-named therapies, most of which are examples of the more general therapies that are claimed, can be found either as entries in Stedman's Medical Dictionary (26th ed., 1995) or are discussed, for example, at page 1311, second column, first full paragraph, of Goldstein, et al. (Clin. Cancer Res. (1995)

1:1311-18, attached hereto as Exhibit B), which cites further references on this topic, indicating that they were well-known in 1995. A person of ordinary skill in the art would have known of the existence of these therapies and how to treat cancer using these therapies at the time the patent application was filed. Thus, there is sufficient support in the parent application for the method claimed in the instant application.

In addition, when defining a "therapeutically effective amount," the specification of the patent application indicates that this term can be applied to a combination, meaning combined amounts of active ingredients (see page 21, lines 31-33), and when describing a "pharmaceutically or physiologically acceptable carrier," the specification notes that supplementary active ingredients can be incorporated into the compositions (see page 20, lines 27-29). Such other active or supplementary active ingredients contemplate those cytotoxins known at the time of filing, including cisplatin, carboplatin, paclitaxel, tamoxifen, taxol, interferon α, and doxorubicin.

Therefore, Applicant respectfully requests that the application be accorded the benefit of the earlier filing date of the parent application 08/532,979, which is September 22, 1995.

5. Claims 1-3 and 7-11 are not anticipated by Tortora et al.

Claims 1-3 and 7-11 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Tortora et al. Applicant respectfully traverses this rejection.

Applicant should be accorded the benefit of the earlier filing date of the parent application 08/532,979, which is September 22, 1995, and thus, this reference will no longer constitute a prior art publication under 35 U.S.C § 102(a). Accordingly, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

However, even if Applicant is not accorded the benefit of the earlier filing date of the parent application 08/532,979, the Tortora et al. reference does not anticipate the instant application. Applicant encloses herewith a Declaration Under 37 C.F.R. § 1.132 and M.P.E.P. § 715.01(c) and § 716.10 by Dr. Sudhir Agrawal, who attests that he is the sole inventor of the invention claimed in the instant application. Dr. Agrawal, who is

one of the authors of the Tortora et al. reference, further attests that the co-authors of the Tortora et al. reference are not inventors of the subject matter which is disclosed and claimed in the instant patent application and described in the publication. Hence, none of the co-authors of the Tortora et al. reference in whole or in part conceived of the subject matter which is disclosed and claimed in the instant patent application and described in the publication. In addition, the Declaration further attests that the co-authors were all working under Applicant's direction using established protocols. Thus, to the extent that the publication is by Applicant, the Tortora et al. reference does not show that the invention was known or used by others in this country, or described in a printed publication, before the invention thereof by the Applicant as required under 35 U.S.C. § 102(a).

Finally, because the filing date of both the provisional application 60/103,098, filed on October 5, 1998, and the parent application 09/022,965, filed on February 12, 1998, are within one year of the November, 1997 publication date of the Tortora et al. reference, the Tortora et al. reference does not anticipate the instant application under 35 U.S.C. § 102(a) or § 102(b).

Accordingly, Applicant respectfully requests that this rejection of claims 1-3 and 7-11 be reconsidered and withdrawn.

6. Claims 1-33 particularly point out and distinctly claim the subject matter which Applicant regards as his invention according to 35 U.S.C. § 112, second paragraph.

Claims 1-33 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicant respectfully traverses this rejection.

The Examiner rejected claims 1, 12, 23, and the claims dependent therefrom as indefinite because the term "capable of" renders these claims indefinite. Claims 1, 12, and 23 have been amended to remove the term "capable of" in order to indicate that the oligonucleotides do down-regulate the expression of nucleic acid encoding protein kinase A subunit RIα. Thus, these amended claims now satisfy § 112, second

paragraph. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

The Examiner rejected claims 1, 12, and 23 because there is insufficient antecedent basis for the limitation "the expression of" in these claims. These claims have been amended to delete the word "the" and correct this problem. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

The Examiner rejected claim 3 because there is insufficient antecedent basis for the limitation "the nucleotide sequence" in claim 1. Applicant respectfully traverses this rejection. The phrase "the nucleotide sequence" in claim 3 refers to "a nucleotide sequence" in the preceding clause of this claim as well as the specific nucleotide sequence set forth in SEQ ID NO: 4. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

The Examiner rejected claims 13-22 because there is insufficient antecedent basis for the limitation "the method." Use of this phrase was a typographical error, and the claims have been amended to correct this error. Thus, these amended claims should now satisfy § 112, second paragraph. Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

Applicant respectfully requests that the rejection of claims 1-33 on the grounds of 35 U.S.C. § 112, second paragraph, be reconsidered and withdrawn.

7. The specification provides enablement for treatment of cancer in vivo.

Claims 1-11 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification does not reasonably provide enablement for treatment of cancer in a patient in vivo. Claims 12-33 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification does not provide sufficient guidance and/or instruction that would allow one of skill in the art to practice the instant methods or use the claimed pharmaceutical compositions throughout the full scope of the claimed invention

without undue experimentation because of the lack of working in vivo examples. Applicant respectfully traverse this rejection.

Examples 27, 28, and 29 (pages 90-95) as well as Figures 16, 17, and 18 provide examples and data indicating that the claimed invention works in vivo. More specifically, Example 27 indicates that HYB 165 inhibits tumor growth after intraperitoneal or oral administration in mice. The data for this experiment is presented in Figures 16A and 16B. Example 28 indicates that oral HYB 165 cooperatively inhibits tumor growth and increases survival in combination with taxol. Data for this experiment is presented in Figures 17A and 17B. Example 29 indicates that the cooperative antitumor effect of HYB 165 with taxol is accompanied by inhibition of new vessel formation and growth factor production as well as other results of histochemical analysis. Data for this experiment is presented in the table in Figure 18. This information clearly indicates that the specification enables the claimed invention for both in vitro and in vivo use by providing supportive data indicating that in vivo use of the invention has, in fact, been achieved.

Additional support for the in vivo use of the methods and pharmaceutical compositions of the invention is found in the description of the figures in the specification at page 20, lines 9-29. Further support for the preferred dosages for the cytotoxic agents and oligonucleotides is found in the specification at page 27, line 15 to page 18, line 14 and page 29, line 8 to page 10.

Accordingly, Applicant respectfully requests that the rejection of claims 1-11 and 12-33 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

CONCLUSIONS

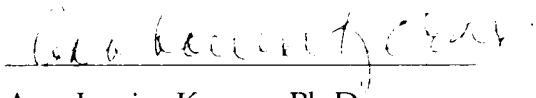
In view of the arguments set forth above, Applicant respectfully submits that the rejections contained in the Office Action mailed on October 24, 2000 have been overcome, and that the claims are in condition for allowance. Applicant also respectfully submits that the instant application should be accorded the benefit of the earlier filing date of the parent application 08/532,979, which is September 22, 1995.

If the Examiner believes that any further discussion of this communication would be helpful, she is invited to contact the undersigned at the telephone number provided below.

Applicant encloses herewith a Petition for a Two-Month Extension of Time pursuant to 37 C.F.R. § 1.136, up to and including March 26, 2001 (March 24, 2001 being a Saturday and March 25, 2001 being a Sunday), to respond to the Examiner's Office Action mailed on October 24, 2000. Our Deposit Account No. 08-0219 is to be charged the $195.00 fee for this purpose.

No other fees are believed to be due in connection with this response. However, please charge any underpayments or credit any overpayments to Deposit Account No. 08-0219.

Respectfully submitted,



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Marked Up Version of Replacement Paragraphs in Specification Under 37 C.F.R. §1.121 (b)(1)(iii)

Cross-Reference to Related Applications:

This application is a non-provisional continuation-in-part application claiming priority from U.S.S.N. 60/103,098, filed on October 5, 1998, and from U.S.S.N. 09/022,965, filed on February 12, 1998, which is a continuation-in-part application of U.S.S.N. 08/532,979 filed September 22, 1995, which issued as U.S. Patent No. 5,969,117.

In the Specification:

Paragraph on pages 1-2, lines 24-36 and 1-6 (Please note that the line numbering on page 1 is incorrect. According to the numbering currently on the page, the line numbers on page 1 are 12-24):

The development of effective cancer therapies has been a major focus of biomedical research. Surgical procedures have been developed and used to treat patients whose tumors are confined to particular anatomical sites. However, at ~~presentation~~ **present**, only about 25% of patients have tumors that are truly confined and amenable to surgical treatment alone (Slapak et al. in Harrison's Principles of Internal Medicine (Isselbacher et al., eds.) McGraw-Hill, Inc., NY (1994) pp. 1826-1850). Radiation therapy, like surgery, is a local modality whose usefulness in the treatment of cancer depends to a large extent on the inherent radiosensitivity of the tumor and its adjacent normal tissues. However, radiation therapy is associated with both acute toxicity and long term sequelae. Furthermore, radiation therapy is known to be mutagenic, carcinogenic, and teratogenic (Slapak et al., ibid.).

Paragraph on pages 3-4, lines 14-32 and 1-9:

One such gene encodes the RI_{α} subunit of cyclic AMP (cAMP)-dependent protein kinase A (PKA) (Krebs (1972) *Curr. Topics Cell. Regul.* **5**:99-133). Protein kinase is bound by cAMP, which is thought to have a role in the control of cell proliferation and differentiation (see, e.g., Cho-Chung (1980) *J. Cyclic Nucleotide Res.* **6**:163-167).

There are two types of PKA, type I (PKA-I) and type II (PKA-II), both of which share a common C subunit but each containing distinct R subunits, RI and RII, respectively (Beebe et al. in *The Enzymes: Control by Phosphorylation*, 17(A):43-111 (Academic, New York, 1986). The R subunit isoforms differ in tissue distribution (Øyen et al. (1988) *FEBS Lett.* **229:**391-394; Clegg et al. (1988) *Proc. Natl. Acad. Sci. (USA)* **85:**3703-3707) and in biochemical properties (Beebe et al. in *The Enzymes: Control by Phosphorylation*, 17(A):43-111 (Academic Press, NY, 1986); Cadd et al. (1990) *J. Biol. Chem.* **265:**19502-19506). The two general isoforms of the R subunit also differ in their subcellular localization: RI is found throughout the cytoplasm; whereas ~~RI~~ **RII** localizes to nuclei, nucleoli, Golgi apparatus and the microtubule-organizing center (see, e.g., Lohmann in *Advances in Cyclic Nucleotide and Protein Phosphorylation Research*, 18:63-117 (Raven, New York, 1984; and Nigg et al. (1985) *Cell* **41:**1039-1051).

Paragraph on page 5, lines 7-18:

Antisense oligonucleotides directed to the RI_α gene have been prepared. U.S. Patent No. 5,271,941 describes phosphodiester-linked antisense oligonucleotides complementary to a region of the first 100 N-terminal amino acids of RI_α which inhibit the expression of RIα in leukemia cells *in vitro*. In addition, antisense phosphorothioate oligodeoxynucleotides corresponding to the N-terminal 8-13 codons of the

RI_{α} gene was found to ~~reduced~~ **reduce** *in vivo* tumor growth in nude mice (Nesterova et al. (1995) *Nature Med.* **1**:528-533).

Paragraph on pages 10-11, lines 22-34 and 1-28:

In another preferred embodiment according to this aspect of the invention, the oligonucleotide is an inverted hybrid oligonucleotide comprising a region of at least four ribonucleotides flanked by 3′ and 5′ deoxyribonucleotide regions of at least two deoxyribonucleotides. The structure of this oligonucleotide is "inverted" relative to traditional hybrid oligonucleotides. In some embodiments, the 2′-O-substituted RNA region has from about four to about ten 2′-O-substituted nucleosides joined to each other by 5′ to 3′ internucleoside linkages, and most preferably from about four to about six such 2′-O-substituted nucleosides. In some embodiments, the oligonucleotides of the invention have a ribonucleotide region **that** comprises at least five contiguous ribonucleotides. In one particularly preferred embodiment, the overall size of the inverted hybrid oligonucleotide is 18. In preferred embodiments, the 2′-O-substituted ribonucleosides are linked to each other through a 5′ to 3′ phosphorothioate, phosphorodithioate, phosphotriester, or phosphodiester linkages. The phosphorothioate 3′ or 5′ flanking region (or regions) has from about four to about 18 nucleosides joined to each other by 5′ to 3′ phosphorothioate linkages, and preferably from about 5 to about 18 such phosphorothioate-linked nucleosides. In preferred embodiments, the

phosphorothioate regions will have at least 5 phosphorothioate-linked nucleosides. One specific embodiment is an oligonucleotide having substantially the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:6. In preferred embodiments of this aspect of the invention, the ribonucleotide region ~~comprise~~ **comprises** 2'-O-substituted ribonucleotides, such as 2'-O-alkyl substituted ribonucleotides. One particularly preferred embodiment is ~~a~~ **an inverted** hybrid oligonucleotide whose ribonucleotide region ~~comprise~~ **comprises** at least one 2'-O-methyl ribonucleotide.

Paragraph on pages 11-12, lines 30-34 and 1-12:

In some embodiments, all of the nucleotides in the inverted hybrid oligonucleotide are linked by phosphorothioate internucleotide linkages. In particular embodiments, the deoxyribonucleotide flanking region or regions has from about four to about 18 nucleosides joined to each other by 5' to 3' phosphorothioate linkages, and preferably from about 5 to about 18 such phosphorothioate-linked nucleosides. In some embodiments, the deoxyribonucleotide 3' and 5' flanking regions of the **inverted** hybrid oligonucleotides of the invention have about 5 phosphorothioate-linked nucleosides. The phosphorothioate linkages may be mixed R_p and S_p enantiomers, or they may be stereoregular or substantially stereoregular in either R_p or S_p form (see Iyer et al. (1995) *Tetrahedron Asymmetry* **6**:1051-1054).

Paragraph on page 17, lines 14-28:

Those skilled in the art will recognize that the elements of these preferred embodiments can be combined and the inventor does contemplate such combination. For example, 2'-O-substituted ribonucleotide regions may well include from one to all nonionic internucleoside linkages. Alternatively, nonionic regions may have from one to all 2'-O-substituted ribonucleotides. Moreover, oligonucleotides according to the invention may contain combinations of one or more 2'-O-substituted ribonucleotide region and one or more nonionic region, either or both being flanked by phosphorothioate regions. (See *Nucleosides & Nucleotides* **14:**1031-1035 (1995) for relevant synthetic ~~techniques.~~ **techniques.)**

Paragraph on page 20, lines 27-29:

FIG. 18 is a tabular representation of histochemical analysis of GEO tumors following treatment with taxol and/or different oral MBOs.

Paragraph on page 21, lines 14-28:

Such synthetic hybrid, inverted hybrid, and inverted chimeric oligonucleotides of the invention have a nucleotide sequence complementary to a genomic region or an RNA molecule transcribed ~~therefore~~ **therefrom** encoding the RI_α subunit of PKA. These oligonucleotides are about 15 to about 30 nucleotides in length, preferably about 15 to 25 nucleotides in length, but most preferably, are about 18 nucleotides long. The sequence of this gene is known. Thus, an oligonucleotide of the invention can have any nucleotide sequence complementary to any region of the gene. Three non-limiting examples of an 18mer of the invention has the sequence set forth below in TABLE 1 as SEQ ID NOS:1, 4, and 6.

Paragraph on page 25, lines 4-14:

The invention also provides therapeutic compositions suitable for treating undesirable, uncontrolled cell proliferation or cancer ~~comprise~~ **comprising** at least one oligonucleotide in accordance with the invention, capable of specifically down-regulating expression of the RI_α gene, and a pharmaceutically acceptable carrier or diluent. It is preferred that an oligonucleotide used in the therapeutic composition of the invention be complementary to at least a portion of the RI_α genomic region, gene, or RNA transcript thereof.

Paragraph on page 25, lines 16-29:

As used herein, a "pharmaceutically or physiologically acceptable carrier" includes any and all solvents (including but **not** limited to lactose), dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Paragraph on pages 25-26, lines 31-34 and 1-6:

Several preferred therapeutic ~~composition~~ **compositions** of the invention suitable for inhibiting cell proliferation *in vitro* or *in vivo* or for treating cancer in humans in accordance with the methods of the invention ~~comprises~~ **comprise** about 25 to 75 mg of a lyophilized oligonucleotide(s) having SEQ ID NOS:1, 4, and/or 6 and 20-75 mg lactose, USP, which is reconstituted with sterile normal saline to the therapeutically effective dosages described herein.

Paragraph on page 26, lines 8-15:

In another aspect, the invention provides pharmaceutical compositions comprising a modified oligonucleotide of the invention in combination with an antibody that binds to epidermal growth factor receptor (EGFR) or a cytotoxic agent. Preferred cytotoxic agents include, without limitation, taxanes, platinum-derived agents, and ~~topoisomeras~~ **topoisomerase** II-selective drugs.

Paragraph on page 28, lines 5-14:

In preferred embodiments according to this aspect of the invention, the first agent is a synthetic modified oligonucletotide having a ~~sequence oligonucleotide has a~~ nucleotide sequence consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4. Preferably, the oligonucleotide is ~~administer~~ **administered** at a dose of up ~~ot~~ **to** 540 mg/m^2/dose by intravenous infusion (2 hours to 21 days in duration or up to 1,050 mg/m^2/day by oral or rectal administration.

Paragraph on pages 28-29, lines 31-34 and 1-6:

A "therapeutically effective manner" refers to a route, duration, and frequency of administration of the pharmaceutical formulation which ultimately results in meaningful patient benefit, as described above. In some embodiments of the invention, the pharmaceutical formulation is administered via injection, sublingually, rectally, intradermally, orally, or enterally in bolus, continuous, intermittent, or continuous, followed by intermittent, regimens.

Paragraph on pages 29-30, lines 8-34 and 1-10:

The therapeutically effective amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the ~~patent~~ **patient** has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the dosages of the pharmaceutical compositions administered in the method of the present invention should contain about 0.1 to 5.0 mg/kg body weight per day, and preferably 0.1 to 2.0 mg/kg body weight per day. When administered

systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of oligonucleotide from about 0.01 μM to about 10 μM. Preferably, the concentration of oligonucleotide at the site of aberrant gene expression should be from about 0.01 μM to about 10 μM, and most preferably from about 0.05 μM to about 5 μM. However, for localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention ~~when~~ **to an** individual as a single treatment episode.

Paragraph on page 30, lines 12-22:

Administration of pharmaceutical compositions in accordance with **the** invention or to practice the method of the present invention can be carried out in a variety of conventional ways, such as by oral ingestion, enteral, rectal, or transdermal administration, inhalation, sublingual administration, or cutaneous, subcutaneous, intramuscular, intraocular, intraperitoneal, or intravenous injection, or any other route of administration known in the art for administrating therapeutic agents.

Paragraph on page 32, lines 10-28:

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile. It must be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as ~~bacterial~~ **bacteria** and fungi. The carrier can be a solvent or dispersion medium. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents. Prolonged absorption of the injectable therapeutic agents can be brought about by the use of ~~the~~ compositions of agents delaying absorption. Sterile injectable solutions are prepared by incorporating the oligonucleotide in the required amount in the appropriate solvent, followed by filtered sterilization.

Paragraph on page 34, lines 20-33:

At least one therapeutic composition of the invention may be administered in accordance with the method of the invention either alone or in combination with other known therapies for cancer such as cisplatin, carboplatin, ~~paclitaxol~~ **paclitaxel**, tamoxifen, taxol, interferon α and doxorubicin. When co-administered with one or more other therapies, the compositions of the invention may be administered either simultaneously with the other treatment(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the compositions of the invention in combination with the other therapy.

Paragraph on pages 36-37, lines 29-34 and 1-21:

The cell line utilized was the CEM-SS cell line (Southern Research Institute-Frederick Research Center, Frederick, MD). These cells are highly susceptible to infection with HIV, rapidly form multinucleated syncytia, and are eventually killed by HIV. The cells were maintained (2-7 x 10^5 cells per ml) in RPMI 1640 tissue culture medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics, and were passaged twice weekly at 1:20 dilution. Passage number was logged each week. Cells were discarded after twenty weeks of passage and fresh CEM-SS cells thawed and utilized in the assay. Stocks of CEM-SS cells were frozen in liquid nitrogen in 1 ml NUNC vials in 90% fetal calf serum and 10% dimethyl sulfoxide (DMSO). Following thawing, CEM-SS cells were routinely ready to be utilized

in the primary screen assay after two weeks in culture. Prior to replacing a late passage cell line, the new CEM-SS cells ~~weree~~ **were** tested in the screening assay protocol utilizing the current stock of infectious virus and AZT. If the infectivity of the virus was significantly different on the new cells or if AZT appeared less active than expected the new cells were not entered into the screening program. Mycoplasma testing was routinely performed on all cell lines.

Paragraph on page 39, lines 25-30:

Titer determinations **included** reverse transcriptase activity assay (see methods below), endpoint titration or plaque assay (CEM-SS) quantification of infectious particles (see methods below), and quantification of cell killing kinetics.

Paragraph on pages 43-44, lines 18-34 and 1-2:

ELISA kits were purchased from Coulter. The assay is performed according to the manufacturer's recommendations. Prior to ELISA analysis we routinely performed the reverse transcriptase activity assays (described above) and used the values for incorporated radioactivity in the RT activity assay to determine the dilution of our samples ~~requires~~ **required** for the ELISA. We have constructed standard curves so that the dilutions of virus to be used in the p24 ELISA can be accurately determined from the RT activity assay. Control curves are generated in each assay to accurately quantify the amount of capsid protein in each sample. Data was obtained by spectrophotometric analysis at 450 nm using a Molecular Devices Vmax plate reader. P24 concentrations were calculated from the optical density values by use of the Molecular Devices software package Soft Max.

Paragraph on page 44, lines 6-25:

Infectious virus particles were ~~qualified~~ **quantified** utilizing the CEM-SS plaque assay as described by Nara, P.L. and Fischinger, P.J. (1988) Quantitative infectivity assay for HIV-1 and HIV-2 *Nature* 332:469-470). Flat bottom 96-well microtiter plates (Costar) were coated with 50 µl of poly-L-lysine (Sigma) at 50 µg/ml for 2 hours at 37°C. The wells were then washed with PBS and 2.5×10^5 CEM-SS cells were placed in the microtiter well where they became fixed to the bottom of the plate. Enough cells were added to form a monolayer of CEM-SS cells in each well. Virus containing supernatant was added from each well of the XTT plate, including virus and cell controls and each serial dilution of the test substance. The number of syncytia were ~~qualified~~ **quantified** in the flat-bottom 96-well microtiter plate with an Olympus CK2 inverted microscope at 4 days following infection. Each syncytium resulted from a single infectious HIV virion.

Paragraph on pages 48-50, lines 27-34, 1-34, and 1-11:

~~[----------~~

~~---------~~To determine the relative effect of inverted hybrid or inverted chimeric structure on oligonucleotide-mediated depletion of complement, the following experiments were performed. Venous blood was collected from healthy adult human volunteers. Serum was prepared for hemolytic

complement assay by collecting blood into vacutainers (Becton Dickinson #6430 Franklin Lakes, NJ) without commercial additives. Blood was allowed to clot at room temperature for 30 minutes, chilled on ice for 15 minutes, then centrifuged at 4°C to separate serum. Harvested serum was kept on ice for same day assay or, alternatively, stored at -70°C. Buffer, or an oligonucleotide sample was then incubated with the serum. The oligonucleotides tested were 25mer oligonucleotide phosphodiesters or phosphorothioates, 25mer hybrid oligonucleotides, 25mer inverted hybrid oligonucleotides, 25mer chimeric oligonucleotides, and 25mer inverted chimeric oligonucleotides. Representative hybrid oligonucleotides were composed of seven to 13 ~~2~~ **2'**-O-methyl ribonucleotides flanked by two regions of six to nine deoxyribonucleotides each. Representative 25mer inverted hybrid oligonucleotides were composed of 17 deoxyribonucleotides flanked by two regions of four ribonucleotides each. Representative 25mer chimeric oligonucleotides were composed of six methylphosphonate deoxyribonucleotides and 19 phosphorothioate deoxyribonucleotides. Representative inverted chimeric oligonucleotides were composed of from 16 to 17 phosphorothioate deoxyribonucleotides flanked by regions of from two to seven methylphosphonate deoxyribonucleotides, or from six to eight methylphosphonate deoxyribonucleotides flanked by nine to ten phosphorothioate deoxyribonucleotides, or two phosphorothioate regions ranging from two to 12 oligonucleotides, flanked by three phosphorothioate regions ranging in size from two to six nucleotides in length. A standard CH50

assay (See Kabat and Mayer (eds), *Experimental Immunochemistry*, 2d Ed., Springfield, IL, CC Thomas, p. 125) for complement-mediated lysis of sheep red blood cells (Colorado Serum Co.) sensitized with anti-sheep red blood cell antibody (hemolysin, Diamedix, Miami, FL) was performed, using duplicate determinations of at least five dilutions of each test serum, then hemoglobin release into cell-free supernates was measured spectrophotometrically at 541 nm.

Paragraph on page 70, lines 3-11:

Two different 18-mer ~~MBO~~ **MBOs** complementary to the RIα subunit of PKAI sequence, HYB 165 and its control oligomer HYB 508, differing only in 4 nucloetide bases, were tested to study their effect on soft agar growth of 1A9 human ovarian cancer cells. While HYB 165 determined a dose-dependent inhibition of colony formation at does ranging between 0.1 and 2.5 μM in all cell lines, the HYB 508 control sequence showed a modest or no growth inhibitory effect. HYB 165 determined an inhibition of 1A9PTX22 cell growth of approximately 5% at a dose of 0.1 μM, of about 50% at 0.5 μM, of about 82% at 1μM and achieved over 95% at 2.5 μM (Fig. 2). Conversely, HYB 508 caused a growth inhibition which at the highest does of 2.5 μM achieved 10%. See Figure 5.

Paragraph on page 90, lines 6-19:

We investigated the antitumor activity of HYB 165 (AS RIα) in nude mice bearing GEO colon cancer ~~xenograts~~ **xenografts**, using either the intraperitoneal (i.p.) or the oral route of administration. When established GEO tumors of approximately 0.2 cm^3 were detectable, groups of 10 mice were treated i.p. with either HYB 165 or a control modified backbone oligonucleotide with a scrambled sequence, at 5 or 10 mg/kg/dose, daily on days 7 to 11 and 14 to 18. Figure 16A shows that i.p. administration of HYB 165 caused a dose-dependent inhibition of growth up to 40% at a dose of 10 mg/kg/dose. The control oligonucleotide produced no inhibition at 10 mg/kg/dose.

Paragraph on pages 91-93, lines 27-35, 1-33, and 1-2:

As illustrated in Figure 17A, treatment with either taxol or the HYB 165 alone inhibited tumor growth as compared to control untreated mice or to mice treated with the scramble MBO. HYB 165 was more effective than taxol, causing over 50% inhibition of tumor size at the completion of the three cycles of treatment. However, shortly after the end of treatment, GEO tumors resumed the growth rate of those in untreated mice or in mice treated with the scramble MBO. When taxol and HYB 165 were used in combination, a marked and sustained inhibition of tumor growth was observed. In fact, tumors of mice treated with taxol and HYB 165 grew very slowly for approximately 60 days following the end of treatment, at which time they resumed a faster growth rate (Figure 17A). Administration of the scramble MBO in combination

with taxol produced an effect ~~simiolar~~ **similar** to that of taxol alone. Within approximately 5 weeks, GEO tumors reached a size not compatible with normal life in all untreated mice and in mice treated with the scramble MBO (Figure 17B). A slight increase in survival time was observed in the group treated with taxol alone, an effect similar to that observed in mice treated with taxol followed by the scramble MBO (data not shown). Treatment with HYB 165 alone also increased survival time as compared to the control group. The delayed GEO tumor growth observed in the group treated with taxol in combination with HYB 165 was accompanied by a prolonged ~~mice~~ **mouse** life span, ~~when,~~ when analyzed with the log-rank test (N. Mantel, *Cancer Chem. Rep.*, 163-170 (1966)), was significantly different as compared to controls ($P < 0.0001$), to the taxol-treated group ($P < 0.0001$) or to the group treated with scramble MBO plus taxol ($P < 0.0001$). In fact, the only mice still alive at 10 weeks after tumor cell injection were those treated with the combination of taxol and HYB 165. Furthermore, about 50% of the mice in this group were still alive after 15 weeks. The combined treatment with taxol and HYB 165 was well tolerated, since no weight loss or other signs of acute or delayed toxicity were observed.

Paragraph on page 93, lines 6-8:

Cooperative antitumor effect of HYB 165 with taxol is accompanied by inhibition of new ~~vessels~~ vessel formation and growth ~~factors~~ factor production.

Paragraph on page 93, lines 10-22:

Tumor specimens from the different groups of mice were examined by histochemical analysis at different time points to evaluate the expression of a variety of biological parameters. ~~Resuts~~ **Results** of the analysis performed on tumor specimens after two cycles of treatment are presented in ~~Table I~~ **Figure 18**. Treatment with HYB 165 inhited expression of the target RIα protein in the tumor. This effect was further increased ~~with~~ **when** HYB 165 was used in combination with taxol. No other treatment was able to affect RIα expression. These results suggest that inhibition of RIα expression is not dependent on growth inhibition.

Paragraph on page 94-95, lines 12-34 and 1-2:

In recent years, the critical role of tumor-induced neovascularization in neoplastic development, progression and metastasis has been elucidated (J. I. Fokman, In: J. Mendelsohn *et al.*, eds., *The Molecular Basis of Cancer*, pp 206-232, Phaladelphia: WB Saunders (1995). A reliable histologic estimate of novel blood vessels on tumor specimens is the microvessel count (MVC) in the most intense areas of neovascularization. In the present study, tumor-induced neovascularization was quantified by immunohistochemistry using an anti-Factor VIII related antigen monoclonal antibody (N. Weidner, *Breast Cancer Res. Treat.*, 36:169-180 (1995)). As shown in ~~Table I~~ **Figure 18**, a significant inhibition of staining was obtained with HYB 165 (about 80%) as well as with taxol (over 60%), as compared to samples from untreated mice or mice treated with the scramble MBO. Combined treatment with taxol and HYB 165 completely suppressed vessel formation in GEO tumors, demonstrating that the cooperative antitumor effect was associated with the marked inhibition of several factors controlling cell cycle, proliferation and angiogenesis of this human colon cancer model.

Marked up Version of Amended Claims in Accordance with 37 C.F.R. § 1.121(c)(1)(ii).

1. (*Once Amended*) A method for inhibiting proliferation of cancer cells comprising

(a) administering to the cells a first agent comprising a synthetic, modified oligonucleotide complementary to, and ~~capable of~~ **which** down-~~regulating the~~ **regulates** expression of, nucleic acid encoding protein kinase A subunit RIα, the modified oligonucleotide having from about 15 to about 30 nucleotides and being a hybrid, inverted hybrid, or inverted chimeric oligonucleotide,

the hybrid oligonucleotide comprising a region of at least two deoxyribonucleotides, flanked by 3′ and 5′ flanking ribonucleotide regions each having at least four ribonucleotides,

the inverted hybrid oligonucleotide comprising a region of at least four ribonucleotides flanked by 3′ and 5′ flanking deoxyribonucleotide regions of at least two deoxyribonucleotides,

and the inverted chimeric oligonucleotide comprising an oligonucleotide nonionic region of at least four nucleotides flanked by two oligonucleotide phosphorothioate regions; and

(b) administering to the cells a second agent comprising an antibody that binds to epidermal growth factor receptor (EGFR) or a cytotoxic agent selected from the group consisting of taxanes, platinum-derived agents, and ~~topoisomeraseII-selective~~ **topoisomerase II-selective** drugs;

wherein the administering steps may be performed simultaneously or sequentially in any order.

2. The method of claim 1, wherein the oligonucleotide is a hybrid oligonucleotide.

3. The method of claim 1, wherein the oligonucleotide has a nucleotide sequence consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4.

4. The method of claim 1, wherein the second agent is an antibody that binds to EGFR.

5. The method of claim 4, wherein the antibody is a monoclonal antibody.

6. The method of claim 5, wherein the antibody is C225.

7. The method of claim 1, wherein the second agent is a taxane.

8. The method of claim 7, wherein the taxane is selected from the group consisting of paclitaxel and docetaxel.

9. The method of claim 1, wherein the second agent is administered prior to administration of the first agent.

10. The method of claim 1, wherein the cancer cells are human cancer cells.

11. The method of claim 10, wherein the human cancer cells are selected from the group consisting of breast cancer cells, colon cancer cells, and ovarian cancer cells.

12. (*Once Amended*) A pharmaceutical composition comprising

(a) a first agent comprising a synthetic, modified oligonucleotide complementary to, and ~~capable of~~ **which** down-~~regulating the~~ **regulates** expression of, nucleic acid encoding protein kinase A subunit RIα, the modified oligonucleotide having from about 15 to about 30 nucleotides and being a hybrid, inverted hybrid, or inverted chimeric oligonucleotide,

the hybrid oligonucleotide comprising a region of at least two deoxyribonucleotides, flanked by 3′ and 5′ flanking ribonucleotide regions each having at least four ribonucleotides,

the inverted hybrid oligonucleotide comprising a region of at least four ribonucleotides flanked by 3′ and 5′ flanking deoxyribonucleotide regions of at least two deoxyribonucleotides,

and the inverted chimeric oligonucleotide comprising an oligonucleotide nonionic region of at least four nucleotides flanked by two oligonucleotide phosphorothioate regions; and

(b) a second agent comprising an antibody that binds to epidermal growth factor receptor (EGFR) or a cytotoxic agent selected from the group consisting of taxanes, platinum-derived agents, and ~~topoisomeraseII-selective~~ **topoisomerase II-selective** drugs.

13. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the oligonucleotide is a hybrid oligonucleotide.

14. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the oligonucleotide has a nucleotide sequence consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4.

15. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the second agent is an antibody that binds to EGFR.

16. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 15, wherein the antibody is a monoclonal antibody.

17. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the antibody is C225.

18. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the second agent is a taxane.

19. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 18, wherein the taxane is selected from the group consisting of paclitaxel and docetaxel.

20. (*Once Amended*) The ~~method~~ **pharmaceutical composition** of claim 12, wherein the second agent is administered prior to administration of the first agent.

23. (*Once Amended*) A method for treating cancer in an afflicted subject comprising

(a) administering to the cells a first agent comprising a synthetic, modified oligonucleotide complementary to, and ~~capable of~~ **which** down-~~regulating the~~ **regulates** expression of, nucleic acid encoding protein kinase A subunit RIα, the modified oligonucleotide having from about 15 to about 30 nucleotides and being a hybrid, inverted hybrid, or inverted chimeric oligonucleotide,

the hybrid oligonucleotide comprising a region of at least two deoxyribonucleotides, flanked by 3′ and 5′ flanking ribonucleotide regions each having at least four ribonucleotides,

the inverted hybrid oligonucleotide comprising a region of at least four ribonucleotides flanked by 3′ and 5′ flanking deoxyribonucleotide regions of at least two deoxyribonucleotides,

and the inverted chimeric oligonucleotide comprising an oligonucleotide nonionic region of at least four nucleotides flanked by two oligonucleotide phosphorothioate regions; and

(b) administering to the cells a second agent comprising an antibody that binds to epidermal growth factor receptor (EGFR) or a cytotoxic agent selected from the group consisting of taxanes, platinum-derived agents, and ~~topoisomeraseII-selective~~ **topoisomerase II-selective** drugs;

wherein the administering steps may be performed simultaneously or sequentially in any order.

24. The method of claim 23, wherein the oligonucleotide is a hybrid oligonucleotide.

25. The method of claim 24, wherein the oligonucleotide has a nucleotide sequence consisting essentially of the nucleotide sequence set forth in SEQ ID NO:4.

26. The method of claim 23, wherein the second agent is an antibody that binds to EGFR.

27. The method of claim 26, wherein the antibody is a monoclonal antibody.

28. The method of claim 27, wherein the antibody is C225.

29. The method of claim 23, wherein the second agent is a taxane.

30. The method of claim 29, wherein the taxane is selected from the group consisting of paclitaxel and docetaxel.

31. The method of claim 23, wherein the second agent is administered prior to administration of the first agent.

32. The method of claim 23, wherein the cancer cells are human cancer cells.

33. The method of claim 32, wherein the human cancer cells are selected from the group consisting of breast cancer cells, colon cancer cells, and ovarian cancer cells.